MATERIALS AND METHODS FOR DETECTION AND TREATMENT OF IMMUNE SYSTEM DYSFUNCTIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a division of application Ser. No. 09/322,628, filed May 28, 1999 now U.S. Pat. No. 6,218,133; which is a division of application Ser. No. 08/916,586, filed Aug. 22, 1997, now U.S. Pat. No. 6,168,792; which is a continuation-in-part of application Ser. No. 08/701,928, filed Aug. 23, 1996, now U.S. Pat. No. 5,939,069.

BACKGROUND OF THE INVENTION

Diabetes is a term that refers to a collection of diseases resulting in disordered energy metabolism and varying degrees of blood glucose elevations or hyperglycemia. One of the best characterized forms of the disease is that which arises from an immunologically mediated destruction of the insulin secreting pancreatic beta cells. This severe form of the disease is termed Insulin-dependent Diabetes (IDD or IMD) since it is associated with progressive insulin deficiency and coincident symptoms such as weight loss, glycosuria and polyuria, and increased thirst or polydipsia. Other terms for this form of diabetes are Type 1 Diabetes (cf. Type 2 Diabetes which results from an inherent resistance to insulin action); Ketosis Prone Diabetes because there is abnormal generation of ketone bodies as a result of excessive breakdown of body fats due to the severe insulin deficiency; or Juvenile Diabetes, since virtually all diabetes that appears in childhood and adolescence is of this type.

Diabetes is a major public health problem, especially in Western countries. The incidence rates vary greatly worldwide, from as high as 40 per 100,000 persons in Finland to as low as 1–2 per 100,000 among the Japanese. The peak incidence is during the pubertal years, associated with the increasing bodily demands for insulin associated with muscle growth. The prevalence rates in the United States population under age 20 years is 0.25% and it approaches 0.4% over a lifetime, albeit an estimated 10–20% of patients with Non Insulin-dependent Diabetes (NIDD) or Type 2 or Maturity Onset Diabetes also have, in reality, slowly progressive IDD. Thus, it is estimated that there may be at least 1 million Americans affected by IDD.

Diabetes results in progressive damage to the blood vessels of the body, to a degree that depends upon the severity of hyperglycemia and its duration. The incident mortality rate for IDD has been calculated to be 7-fold higher than for age matched non-diabetic controls. Whereas the decade long Diabetes Control and Complications Trial (DCCT)—concluded in 1994 by the National Institutes of Health in the United States—showed that meticulous insulin replacement therapy would slow the appearance of damaged arteries, it was not able to completely prevent this damage since blood glucose levels were difficult to keep within normal limits. Ocular complications of diabetes are the leading cause of new blindness in persons 20–74 years of age. The risk of lower extremity amputation is 15-fold higher in those with diabetes. Approximately 40% of persons undergoing renal transplantations have kidney failure because of diabetes, and the proportion due to diabetes continues to rise each year. Women with diabetes produce newborn infants with a 7% newborn mortality rate. Other complications of diabetes include increased heart disease and stroke, loss of nerve cells or neurons enervating the limbs and intestine, impotence and infertility, cataract formation in the lens of the eyes, increased periodontal disease, and predisposition to infectious diseases especially from bacteria and yeast. Of all patients with diabetes, those with IDD have a disproportionate share of these complications because of its severity and usual early age of onset. In the United States, the direct health care costs attributable to diabetes in 1994 have been estimated to exceed $120 billion. Thus it is important that the pathogenesis of IDD be understood and strategies be developed to prevent it as a fully expressed clinical disease.

Patients with IDD are unusually prone to other diseases that have become recognized as having autoimmune origins. These diseases include thyroiditis or Hashimoto's disease, Graves' disease, Addison's disease, atrophic gastritis and pernicious anemia, celiac disease, and vitiligo (Maclaren, N. K. [1985] *Diabetes Care* 8(suppl.):34–38). Evidence that IDD itself has an autoimmune nature began with histological studies of patients; these studies indicated that the islets were infiltrated with a chronic inflammatory (lymphocytic) infiltrate termed insulitis. This was supported in the early 1970s by reports of islet cell autoantibodies reactive to antigens within the cytoplasm (ICA) (Lendrum et al. [1975] *Lancet* 1:880–882) or confined to the islet cell surfaces (ICSA) (Maclaren et al. [1975] *Lancet* 1:977–1000) as detectable by indirect immunofluorescence. Later it was recognized that many patients also develop autoantibodies to insulin (IAA) before their diagnosis (Palmer et al. [1983] *Science* 222:1337–1339) as well as to insulin receptors (Maron et al. [1983] *Nature* 303:817–818). Autoantibodies were also reported to an islet cell protein composition of 64,000 M.Wt. in man (Baekkeskov et al. [1982] *Nature* 298:167–169), in the Biobreeding (BB) rat model (Baekkeskov et al. [1984] *Science* 224:1348–1350), and in the Non Obese Diabetic (NOD) mouse model (Atkinson and Maclaren [1988] *Diabetes* 37:1587–1590). 64 kDa antigen has subsequently been reported to be the lower molecular weight isoform of glutamic acid decarboxylase (GAD_{65}) (Baekkeskov et al. [1990] *Nature* 347:151–156) (Kauffman et al. [1992] *J. Clin. Invest.* 283–292). GAD is an enzyme that converts glutamate into the membrane stabilizing neurotransmitter called gamma amino butyric acid or GABA. In addition to autoantibodies to GAD, peripheral blood mononuclear cells were shown to be autoreactive in patients developing IDD (Atkinson and Maclaren et al. [1992] *Lancet* 339:458–459; and Harrison et al. [1993] *Lancet* 341:1365–1369).

It has previously been demonstrated in several autoimmune diseases, including IDD, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis (MS), and autoimmune thyroid disease, that antigen-presenting cells (APCs) such as monocytes and macrophages are dysfunctional in their ability to activate T lymphocytes (Via, C. S. et al. [1993] *J. Immunol.* 151:3914–3922; Serreze, D. [1993] *FASEB J.* 7:1092–1096; Rasanen, L. et al. [1988] *Clin. Exp. Immunol.* 71:470–474; Hafler, D. A., et al. [1985] *J. Neuroimmunol.* 9:339–347). The defect(s) in APC function, however, have thus far not been defined at the cellular or molecular level.

Prostaglandins (PGs) are lipid molecules formed from a precursor molecule, arachidonic acid, through the actions of specific enzymes called prostaglandin synthases (PGS-1 and PGS-2). PGS-1 mRNA and protein are constitutively expressed, and this enzyme is responsible for the production of low levels of PGs and functions as a housekeeping molecule. PGS-2 is an inducible enzyme expressed by macrophages and monocytes during inflammation and following exposure to mitogens, cytokines, and bacterial cell